Unmethylated A vector

replicative   Active copy of
genes   marker gene   X

Inactive copy of   Y
marker gene

Methylated I Vector

Anneal and ligate

marker gene   *test sequence*

*Heteroduplex DNA having
one methylated strand*

*Transform bacterial host
Plate transformants*

*Perfect match
between X and Y*

*Mismatch present
between X and Y*

*Colonies express
active marker gene*

*Colonies lack
active marker gene*

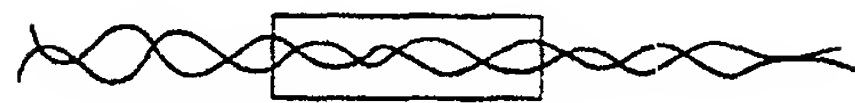**FIG. 1**

Genomic copy Y sequence

PCR amplification

Denature

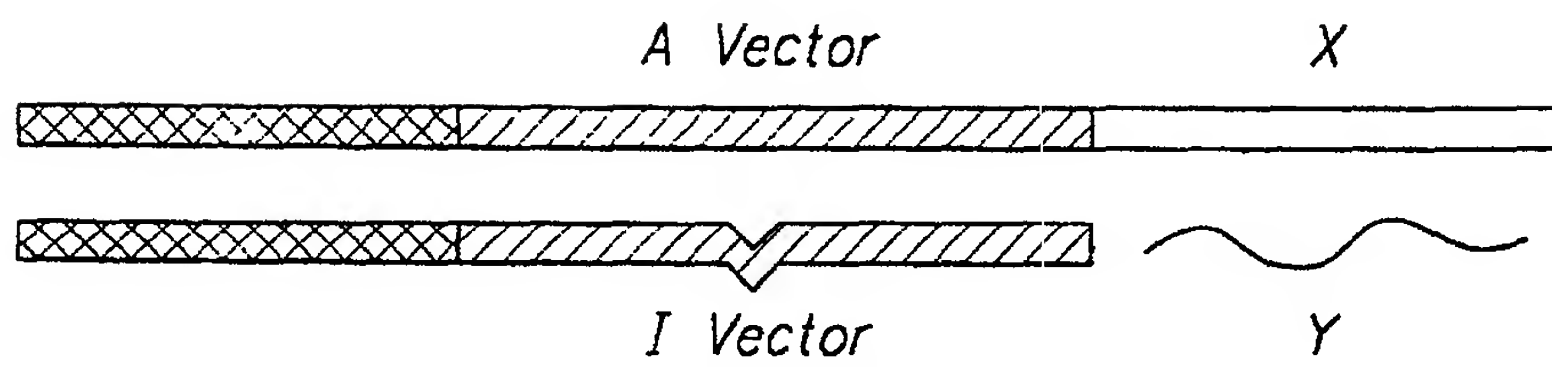Hybridize vectors and Y strand.
Ligate.

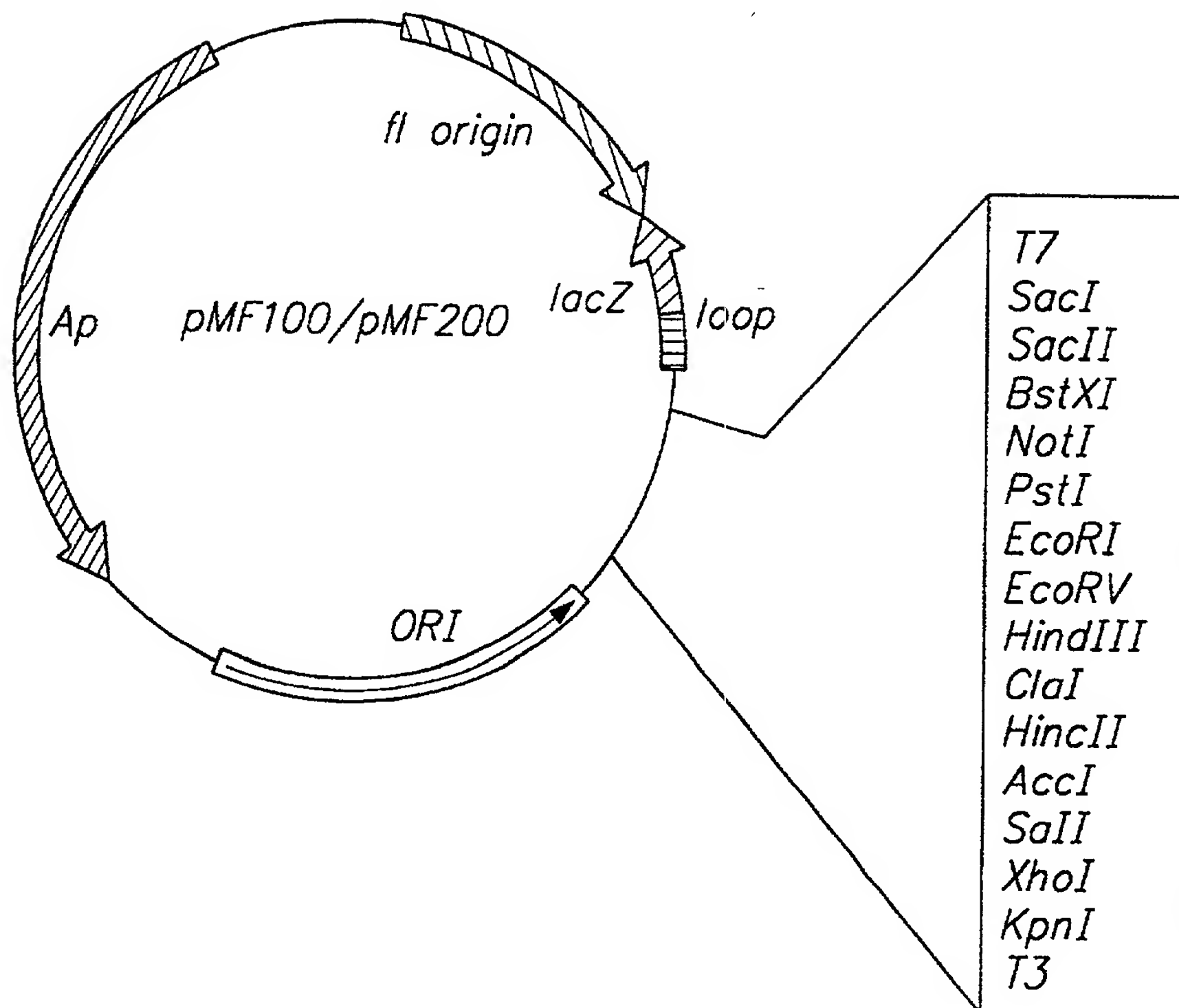A Vector   X
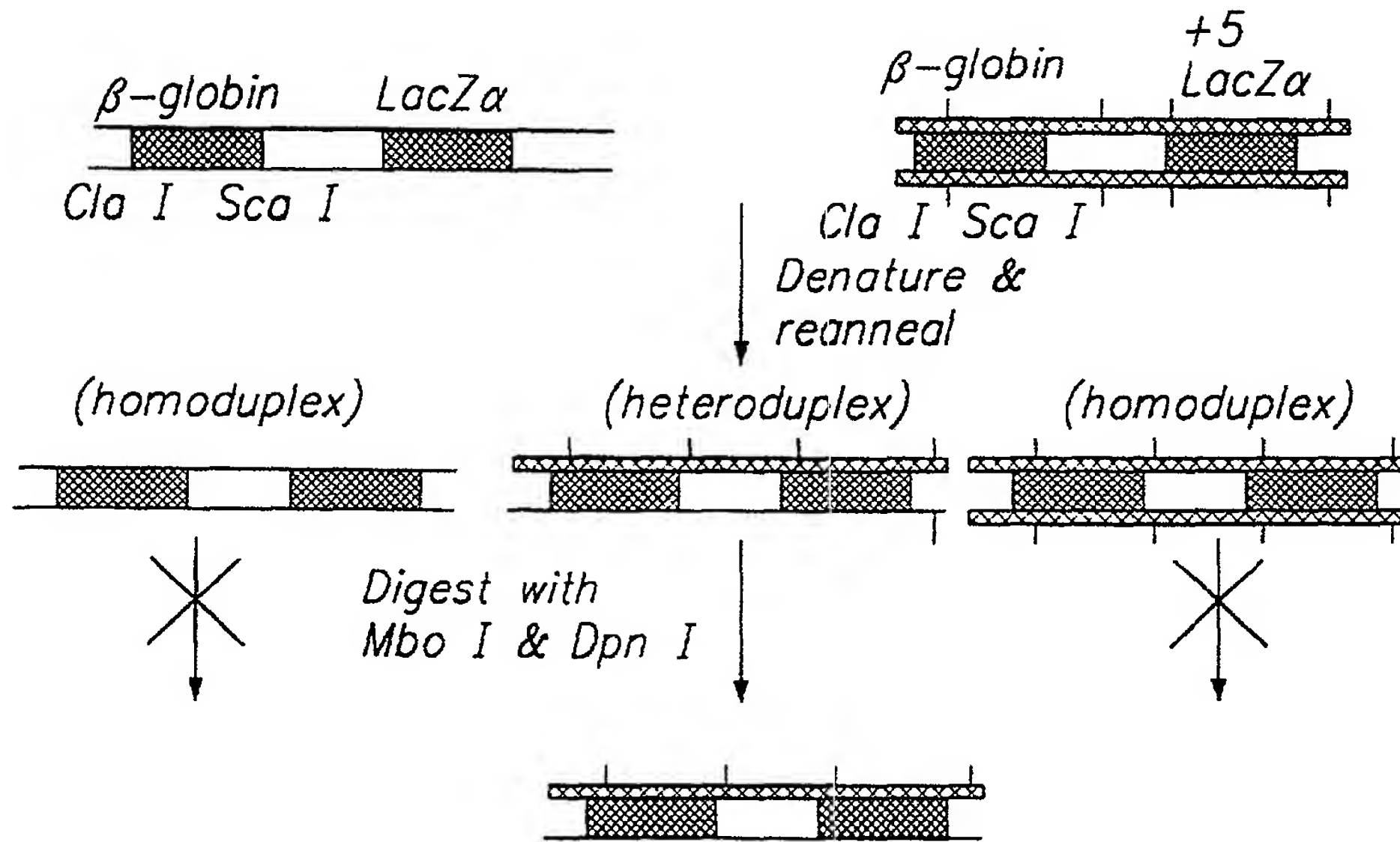
I Vector   Y

FIG. 2

fl origin

Ap

pMF100/pMF200

lacZ loop

ORI

T7
SacI
SacII
BstXI
NotI
PstI
EcoRI
EcoRV
HindIII
ClaI
HincII
AccI
SaII
XhoI
KpnI
T3

FIG. 3

FIG. 4

pick blue colonies*

Isolate DNA

pick white colonies*

Isolate DNA

Use as a hybridization probe
on a dot blot filter containing
the fragments tested

Use as a hybridization probe
on a dot blot filter containing
the fragments tested

comparing the hybridization signal on both filters,
one can determine which fragments are variant

FIG. 5

Make heteroduplexes

1) treat with mung bean
nuclease then Taq ligase
or
treat with Taq ligase
then exnuclease III

2) Transform into MS strain

FIG. 6A

*In absence of a variation,
no repair occurs.
Both strands are replicated*

*In presence of a variation,
repair occurs. Only the
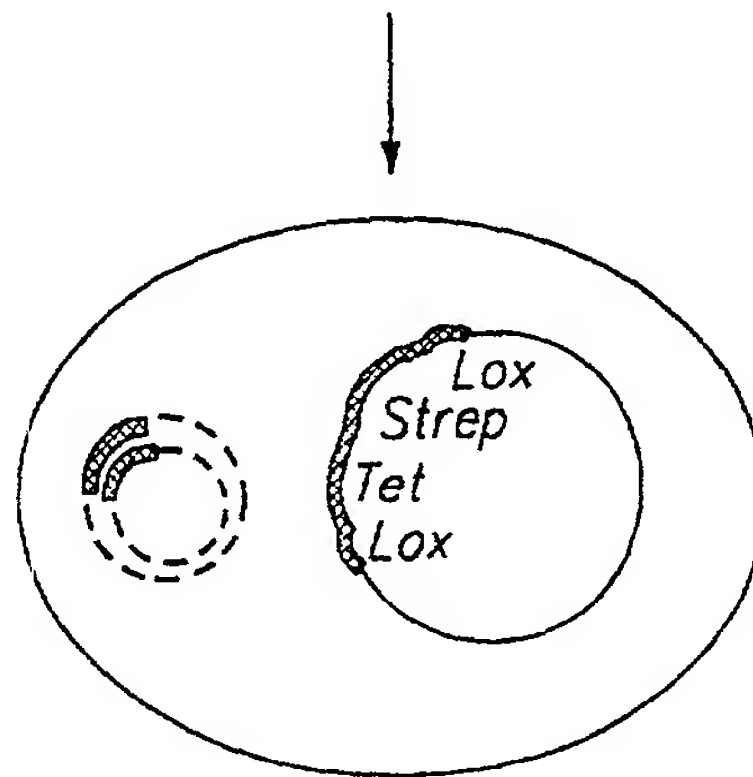strand w/inactive Cre is replicated*
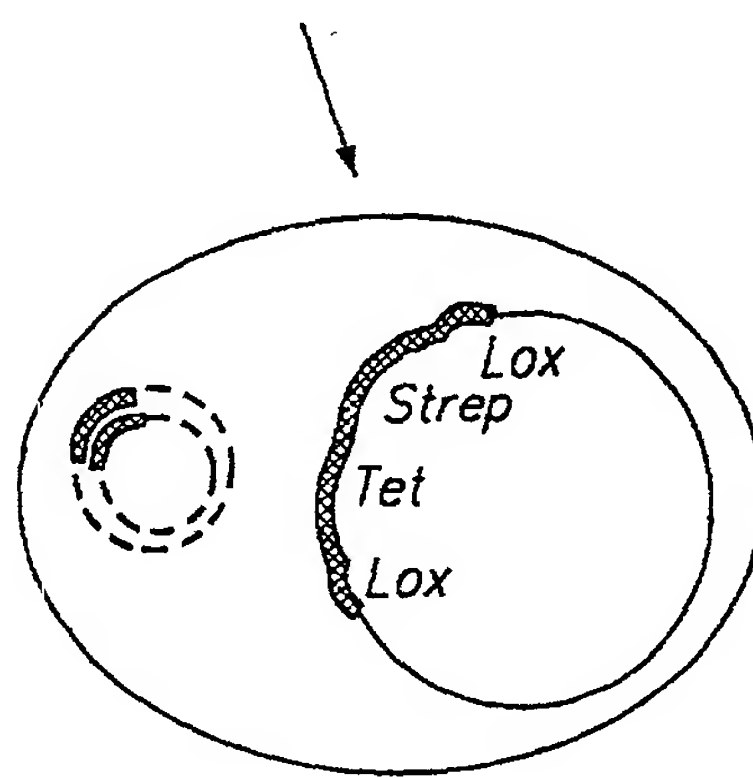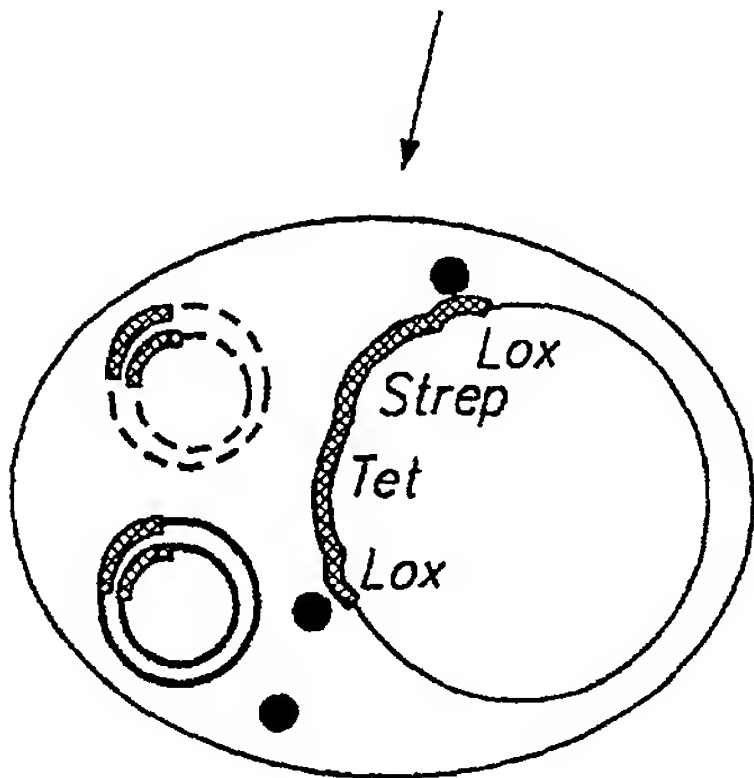
Active Cre is present in the cell

Active Cre is absent in the cell
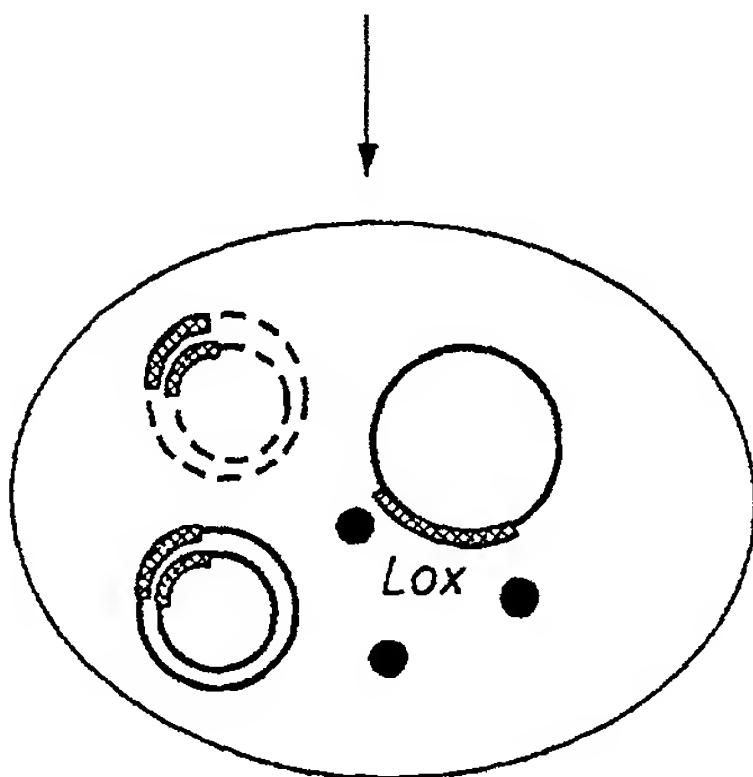
*Cell is Tet sensitive
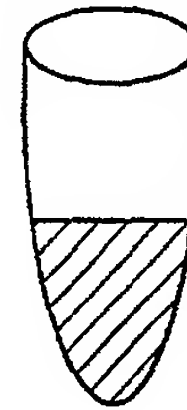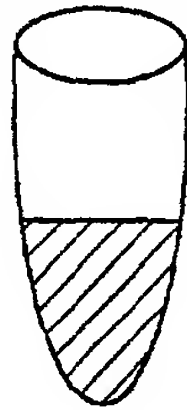& Strep resistant*

*Cell is Tet resistant
& Strep sensitive*

FIG. 6B

Cells are grown in two tubes supplemented either with
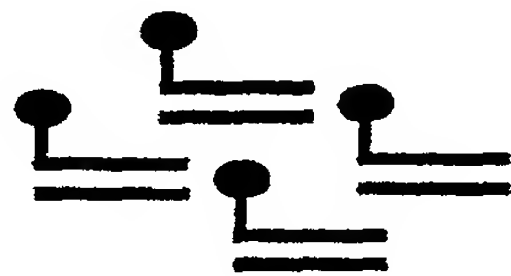
Tetracycline   or   Streptomycin

Next day DNA is preped from the pool of the cells
grown in each tube

DNA from the Tet pool is
labeled with green fluorescence

DNA from the Strep pool is
labeled with red fluorescence

DNA from both pools are mixed and hybridized
to a DNA microarray.
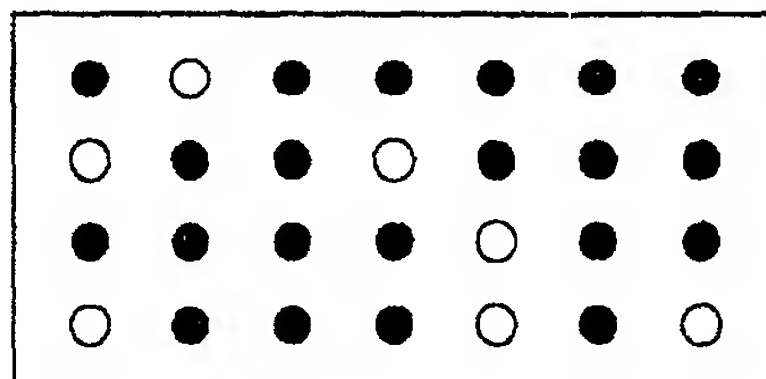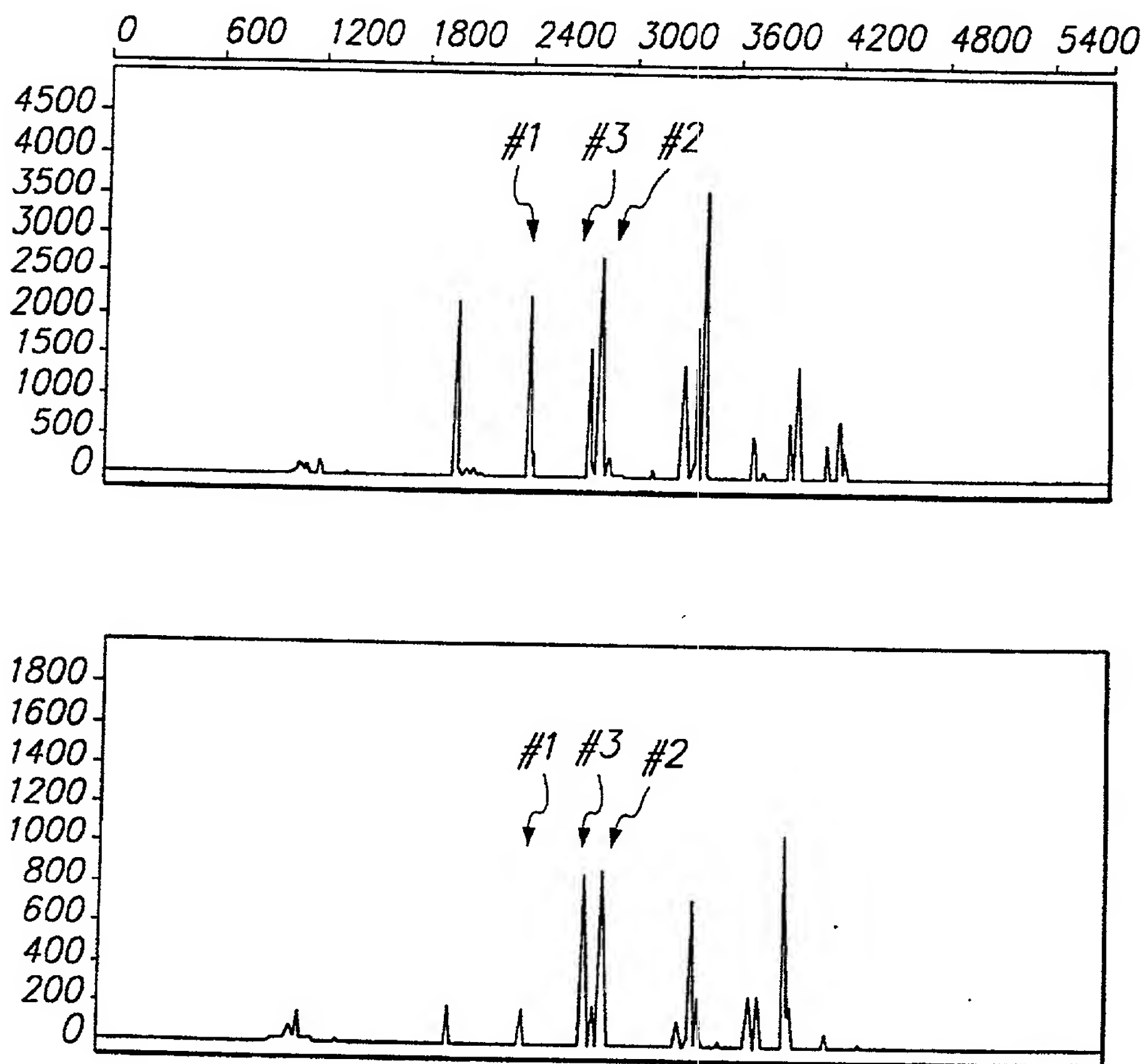Each spot corresponds to a different gene fragment
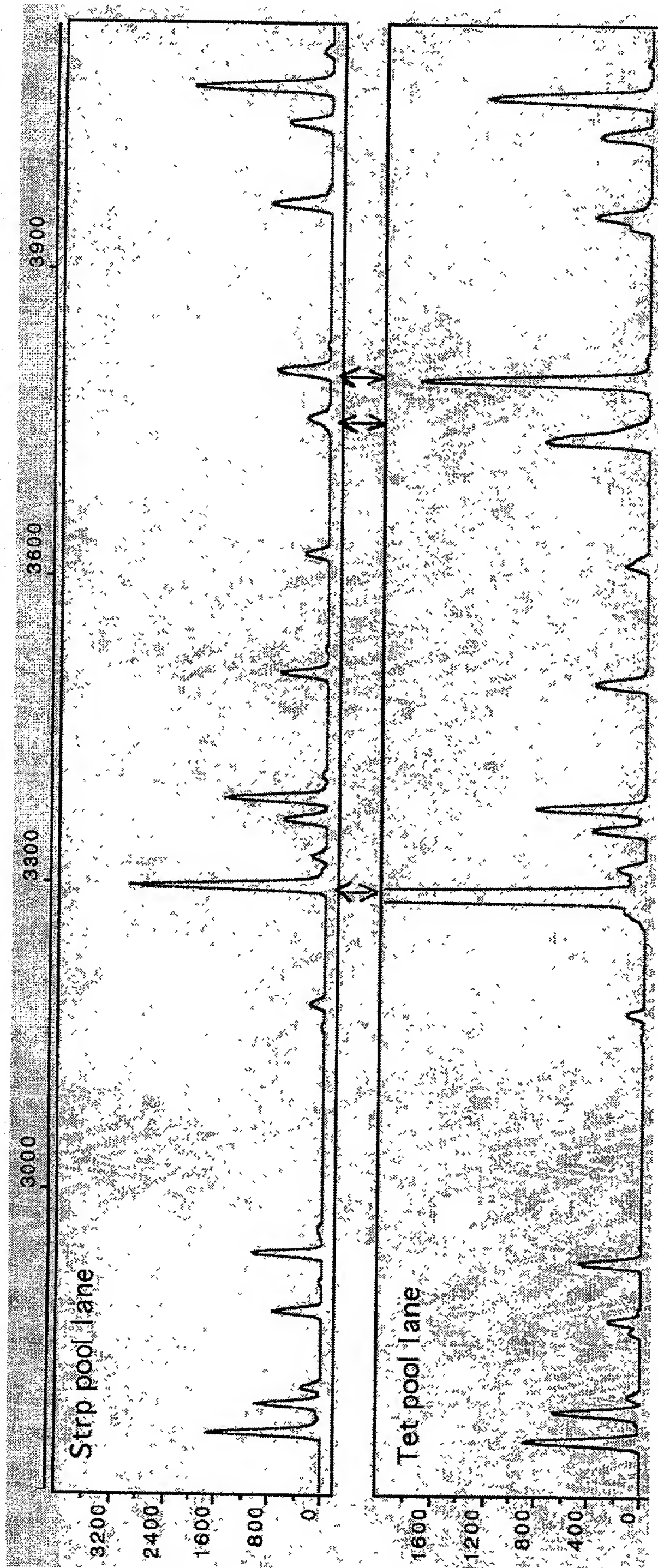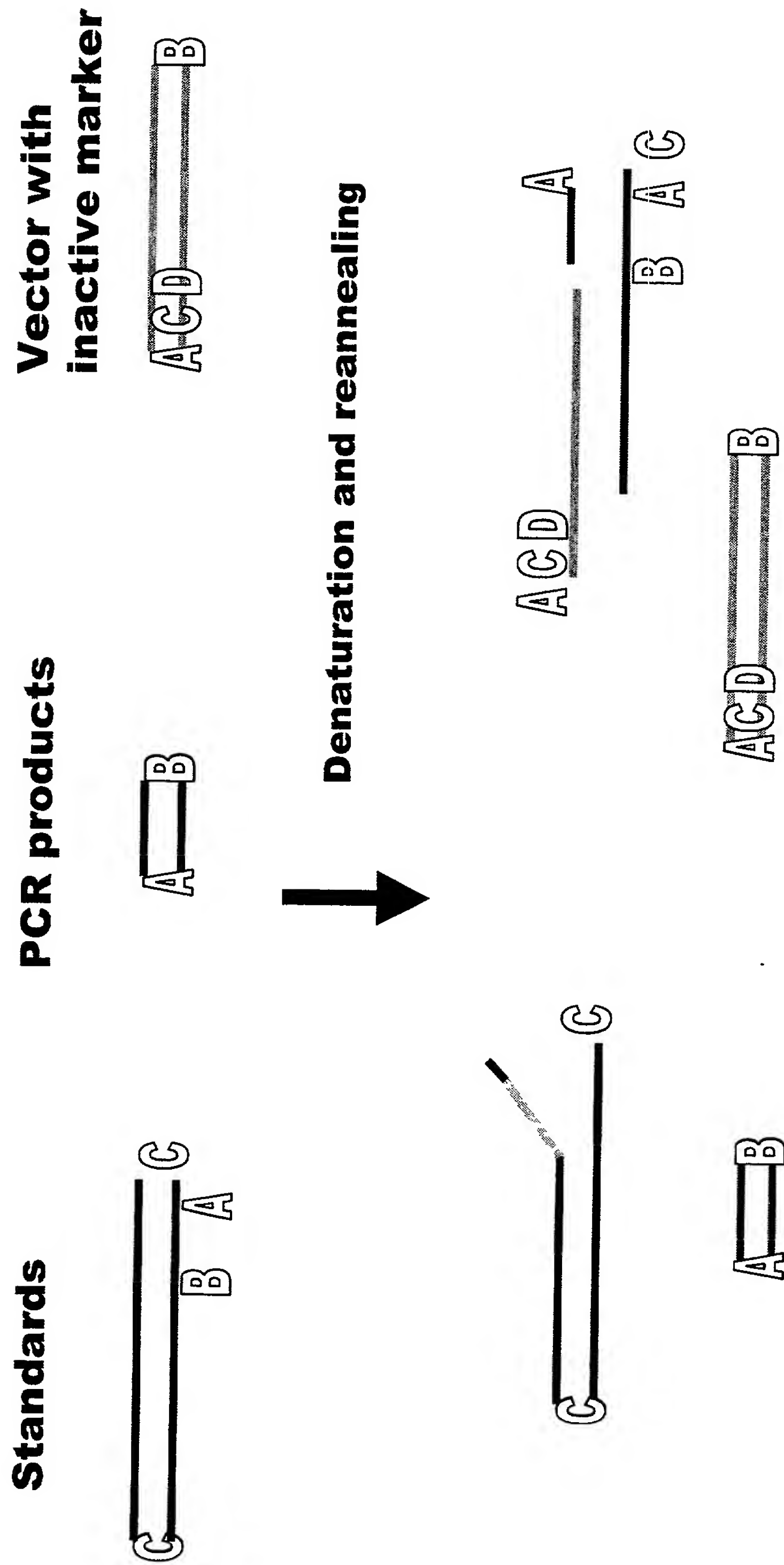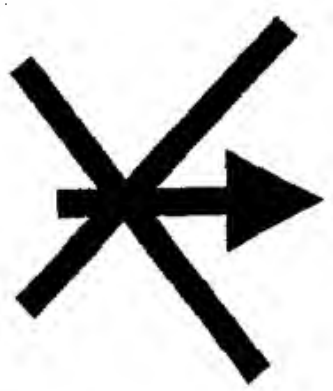that is being tested

FIG. 6C

FIG. 7

FIG. 8

FIG. 9A

elimination of wholly or
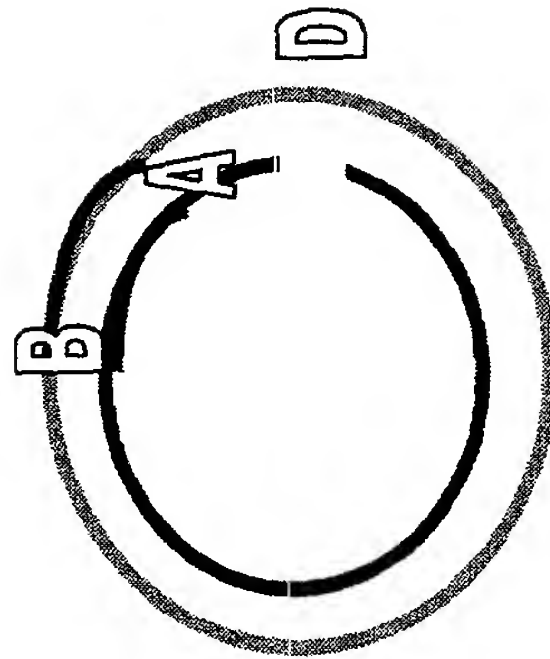partially single stranded
species

ligation of compatible ends

FIG. 9B
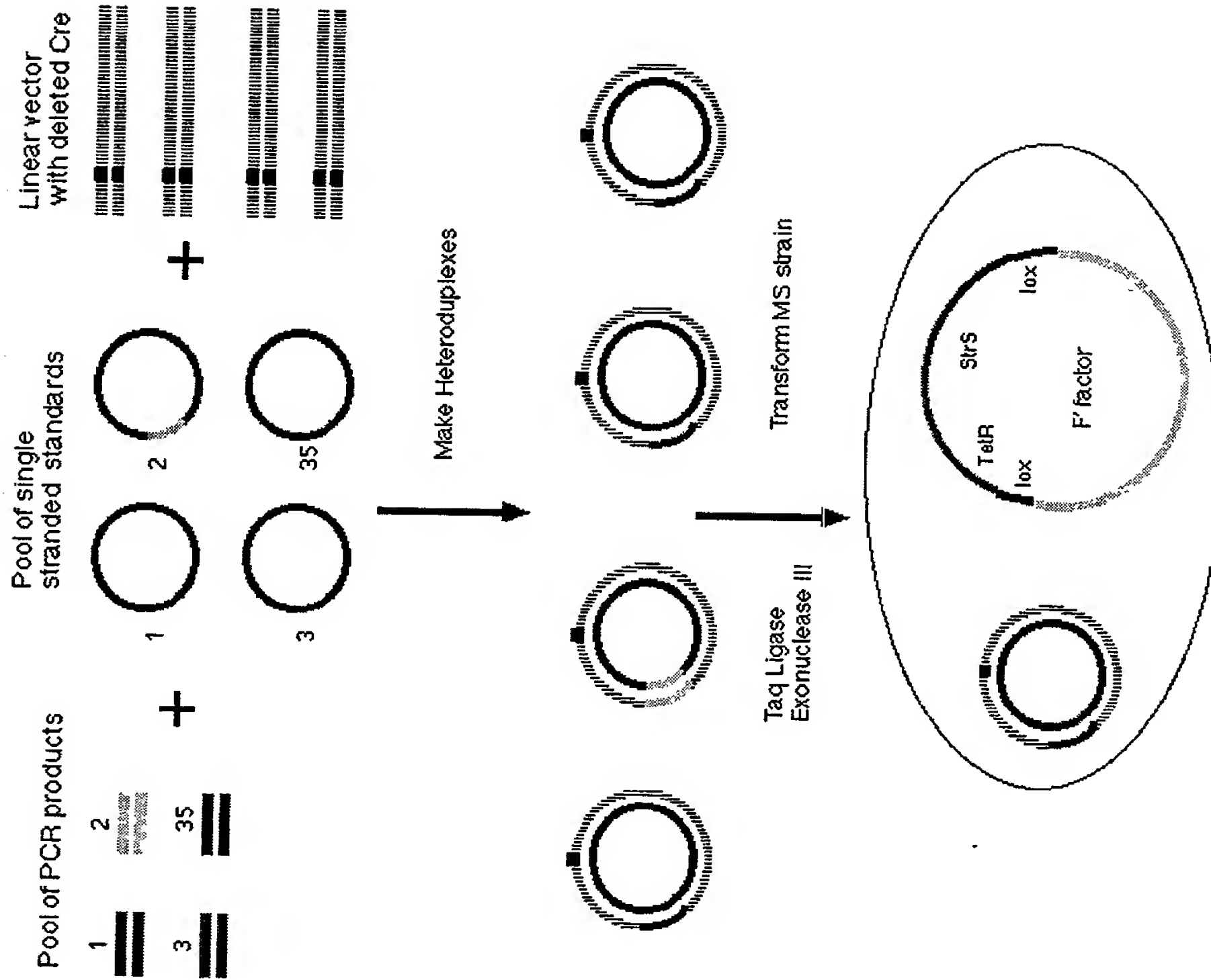
FIG. 10A

FIG. 10B

FIG. 10C

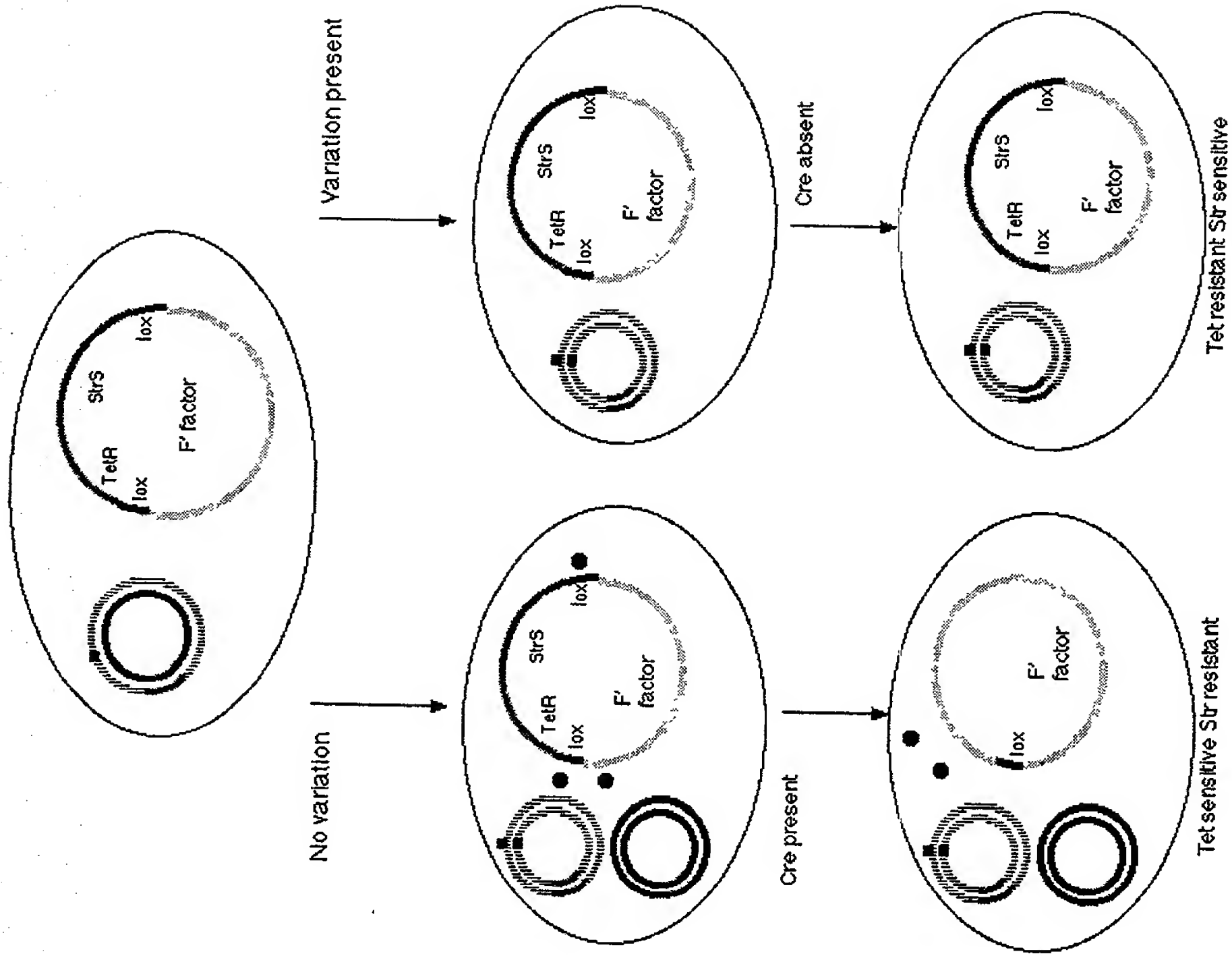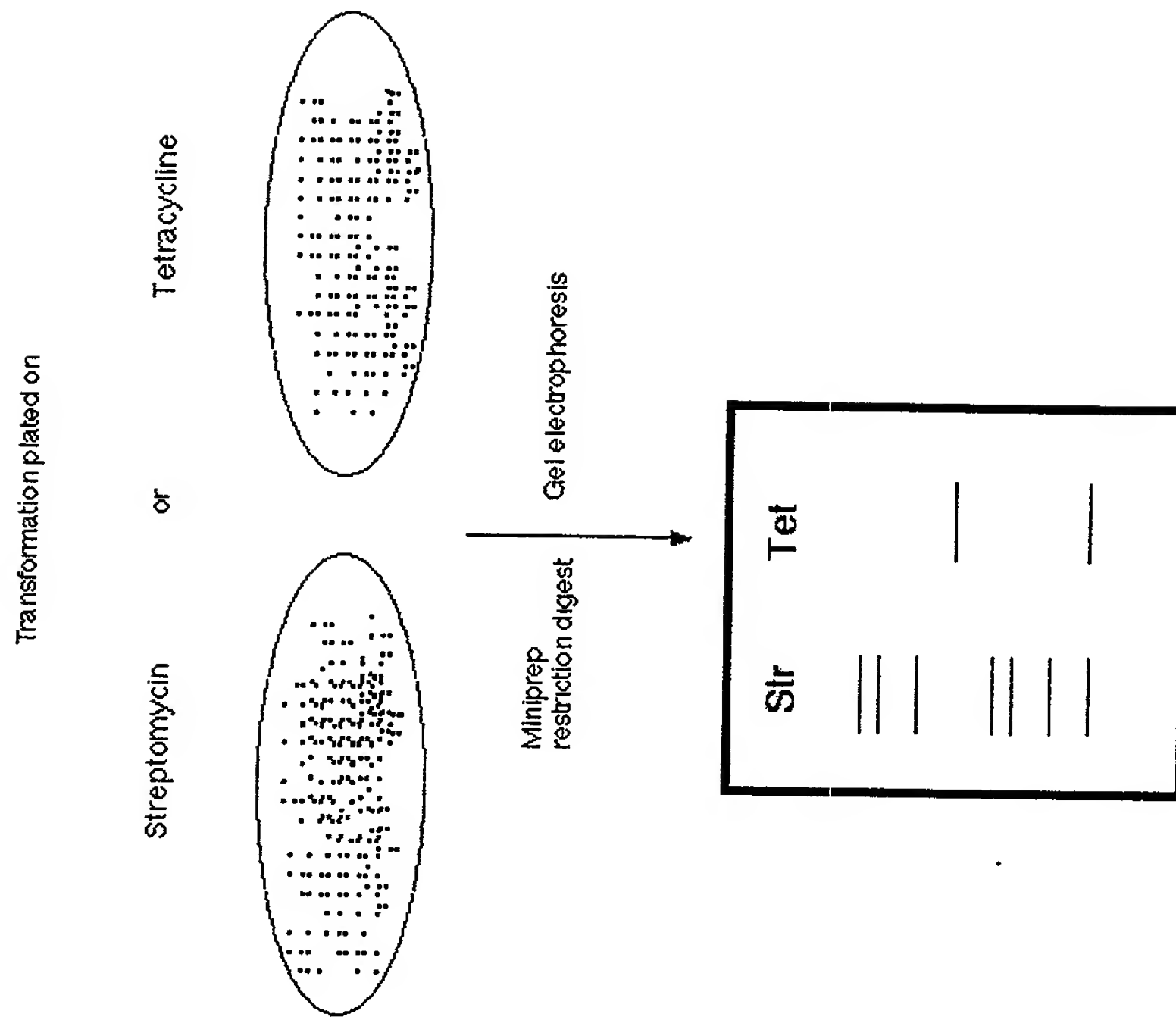**FIG. 11**